Sektion 1: Herbizidresistenz – Methoden

Section 1: Herbicide resistance - methods

Framework for an expert evaluation for the evolution of weed resistance

Rahmen für eine Experten-Bewertung für die Entwicklung der Unkrautresistenz

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Abstract

The apparent rate of evolution of resistance of weeds to herbicides has increased substantially over the past decade. Data suggests phenotypic expression is affected by the mechanism of action of the herbicide, the taxonomy of the weed, the extent and frequency of selection and the agronomic context of herbicide use that contribute to the development of herbicide resistance. The opportunities for herbicide resistance are maximized in weeds that produce prolific seed, have relatively short half-life in the seedbank, and are obligate outcrossers; for herbicides that have a single target site that is not conserved or have multiple non-target resistance mechanisms; and in management, those management systems that do not use a diverse set of weed management tools and rely only on herbicides for weed control. The ability to identify weed and herbicide combinations that are most likely to develop herbicide resistance can aid in education and management systems to delay herbicide resistance.

Keywords: Herbicide resistance, resistance management, resistance Index, integrated weed management

Zusammenfassung

Die Herbizidresistenz von Unkräutern hat in den letzten zehn Jahren deutlich zugenommen. Versuchsergebnisse weisen darauf hin, dass die Entwicklung der Herbizidresistenz von dem Wirkmechanismus der Herbizide, der Taxonomie der Unkräuter, dem Ausmaß der Selektion und den agronomischen Verhältnissen beeinflusst wird. Die Gefahr einer Herbizidresitenz nimmt bei Unkrautarten zu: mit hoher Samenproduktion, relativ niedriger Überlebensrate in der Bodensamenbank, obligater Auskreuzung, bei Unkräutern mit einem Zielort, mit einer Nichtzielort-Resistenz, einseitiger Unkrautbekämpfung. Es ist erforderlich Ursachen zu identifizieren, die die Herbizidresistenz fördern. Dies kann dazu beitragen durch Änderung von Managementsystemen die Entwicklung der Herbizidresistenz zu verzögern.

Stichwörter: Herbizidresistenz, Resistenzmanagement, Resistenzindex, integrierte Unkrautbekämpfung

Introduction

The number and diversity of herbicides available for use in agriculture has seen tremendous growth since the introduction of 2,4-D in the 1940's (Appleby, 2005). From the beginning of herbicide use on a large scale, concerns about the potential development of herbicide-resistant biotypes among agricultural weed populations have been expressed (Appleby, 2005). These fears were realized in 1968 when the first case of triazine resistance was confirmed (Ryan, 1970). Since that time, over 403 resistant weed biotypes have been reported within all major herbicide mechanisms of action (Heap, 2013).

Many herbicides are registered for use in row crops; however herbicides are grouped by chemists and weed scientists into a relatively small number of classifications based on their mechanism of action, that is, by reference to the biochemical pathways that they disrupt in susceptible plants (Ashton and Crafts, 1981). Frequently, herbicides with the same mechanism of action control approximately the same botanical families. Thus, whenever they are used in weed management

programs, they tend to exert selection against the same groups of weed species. Since the introduction of glyphosate-resistant cultivars in the mid-1990's, herbicide application patterns have changed dramatically. This has resulted in weed control programs with less diverse mechanisms of action and intensifying selection pressure for herbicide resistance, particularly glyphosate.

Herbicide resistance has become a critical issue to growers in the developed world such as North America, Europe and Australia. There have been no new herbicide mechanisms of action introduced since 1992 and multiple-resistant weeds are reducing the number of herbicides available to control some of the most common and troublesome weeds in certain cropping areas such as *Amaranthus* in North America, *Alopecurus* in Western Europe, and *Lolium* in Australia (VENCILL *et al.,* 2012). There are newer herbicide-resistant crop technologies being developed such as auxinic-resistant cotton and soybean. Under this scenario, it would be most beneficial to weed scientists and others to have a better understanding of what herbicide-weed-management scenarios; herbicide-resistant weeds are most likely to develop.This paper will present a framework for determining the potential for herbicide resistance development under given weed, herbicide, and management conditions.

Weed Factors

Many factors determine the success of a weed (e.g. competitiveness, emergence pattern, seed production potential etc.). For example, annual weeds develop resistance more quickly than biennial or perennial species because more generations experience selection over the same period. Perennial weeds, if predominantly vegetatively propagated, are much less likely to evolve resistance than annual weeds. The weed biology risk matrix described below is primarily for annual weeds, although it will also be relevant for perennial weed species if propagation by seeds is important.

Seedling emergence probably is the single most important phenological event that influences the success of an annual plant. In theory, a persistent seed bank of older, less selected seeds, could 'buffer', and hence reduce, the effect of selection for resistance (GRESSEL, 2009). However, due to limited knowledge of the interaction of seed bank dynamics and resistance, there are no documented cases of this occurring. In contrast, once resistance has evolved, species with more persistent seeds are likely to pose a *greater* threat due to long-term emergence of resistant plants from seeds in the seedbank because of a similar buffering effect.

Differing weed species have a wide range of seed production. In theory, a weed that set more seed would have a greater chance of developing herbicide resistance due to a greater number of genetic combinations that have the potential to produce an individual with a herbicide-resistance trait. Several common and troublesome weeds are displayed in Table 1. It shows that many of the weed species with the highest fecundity have the greatest number of herbicide resistant biotypes (e.g. *Amaranthus, Echinochloa*).

Genetic diversity is a key factor in the development of herbicide resistance. Genetically diverse species are more likely to contain individuals that already possess herbicide resistance. Weed species that ensure greater genetic diversity through obligate outcrossing tend to be some of the weeds with the greatest propensity for herbicide resistance development (Tab. 1).

Pollen and seed dispersal affect how widely an evolved trait of resistance can move once it has developed. The pollen for some weeds such as *Amaranthus palmeri* (Palmer amaranth) have been shown to move great distances allowing pollen carrying resistance genes to infest a greater geographic area (SOSNOSKIE *et al.,* 2009). Weeds such as horseweed (*Conyza canadensis*) that have wind-blown propagules can spread an evolved herbicide resistance trait over a large geographic distance (DAUER *et al.,* 2007).

Tab. 1 Biological characteristics of selected common and troublesome agronomic weed species (VENCILL, 2012).

***Tab. 1** Biologische Eigenschaften von ausgewählten Unkrautarten (VENCILL, 2012).*

Weed	Dioecious	Obligate outcrosser	Fecundity	Seedbank longevity	Resistance Cases	Rank[a]
			seed plant^{-1}	yr	#	
Amaranthus palmeri	Yes	No	1,000,000	3	25	3
Amaranthus tuberculatus	Yes	No	1,000,000	3	37	3
Alopecurus myosuroides	Yes	No	3000	1-2	26	3
Ambrosia artemisifolia	No	No	6000	1	19	2
Ambrosia trifida	No	Weak	1400	4	18	2
Avena fatua	No	Yes	1000	3	39	2
Chenopodium album	No	Yes	72000	4	42	1
Echinochloa crus-galli	No	Yes	500000	5	31	3
Ipomoea spp.	No	Yes	1500	1	0	1
Kochia scoparia	No	Yes	4100	2	34	2
Lolium rigidum	Yes	No	2000	1-2	44	3
Poa annua	No	Yes	1000	2	21	3
Senna obtusifolia	No	Yes	200	>5	0	1
Xanthium strumarium	No	Yes	9000	2	17	2

[a]Ranking on a scale of 1-3 with one having the least risk and three the greatest risk of resistance development.

Annual weed species display a wide range of emergence patterns from those that emerge over a narrow time frame to those that have continuous germination over the season. Those weeds that germinate continuously over a season would develop herbicide resistance faster than those who do not due to a greater exposure of the weed seedbank over time to any given herbicide. In addition, and greater phenotypic diversity is present in weeds that germinate over the season.

Herbicide Factors

The herbicide factors that contribute to herbicide-resistance are related to herbicide use patterns, weed control spectrum, and interaction of the herbicide with the target site. Herbicides that provide a high level of control at recommended rates are likely to select for highly resistant individuals more effectively than herbicides with lower intrinsic activities, where a higher proportion of susceptible plants are likely to survive too (GRESSEL and SEGEL, 1990). The intrinsic activity of the herbicide may be more important to selection for target site resistance than for enhanced metabolism. For example, MANALIL *et al.* (2011) showed low herbicide doses increased non-target site resistance in *Lolium rigidum*.

Greater residual activity might, in theory, increase resistance risk by exposing successive flushes of emerging weeds to the herbicide. In practice, there is little evidence that this is of great importance. In situations where most weeds have emerged before application, this factor will not be relevant. Although two (ALS, triazines) herbicide classes in the high risk category in the figure above have residual activity, it is not proven that this is a significant factor in relation to resistance. Residual activity may have more relevance, where high rates of persistent herbicides may be used.

There are many factors required for a herbicide to successfully control a target weed. If foliar applied, it must be able to cross the plant cuticular barrier and be translocated to a cellular target site. If soil applied, it must be available in the soil solution in sufficient quantities to be taken up by roots and translocated to a cellular target site. Differences in crops and weeds and among weeds to the same herbicide can lead to selectivity or to the development of resistance in certain cases

such as glyphosate-resistant *Conyza* or *Lolium*. There are many ways herbicides interact with their primary target sites. Some herbicides are competitive inhibitors (they compete with the natural substrate at the target site for the herbicide). In these situations, changes to the target site that would lead to target site herbicide-resistant weeds are more difficult to develop as many of these target sites are highly conserved such that many of the changes to the target site that would reduce a given herbicide's binding would also reduce the binding of a natural substrate leading to a lethal mutation. For example, photosystem II inhibitors (triazines and substituted-ureas), EPSPS-inhibitors (glyphosate), glutamine-synthetase inhibitors (glufosinate), PPO-inhibitors (diphenyl ethers), phytoene-desaturase inhibitors (pyridazinone herbicides), and HPPD-inhibitors such as the triketone herbicides are competitive with the natural substrate for target site for the herbicide (VENCILL *et al.,* 2012). This was one of the reasons that weed scientists initially thought glyphosate-resistant weeds would be rare (SAMMONS *et al.,* 2007). However, glyphosate-resistance has developed through non-target site resistance mechanisms such as enhanced metabolism, sequestration, or amplified target site. However, we did not take fully into account the myriad of non-target site resistance mechanisms that could be selected for in a weed population. Other herbicides such as the ALS-inhibitors and ACC-ase inhibitors are not competitive inhibitors. For these herbicide target sites, a mutation to the target site leading to herbicide resistance is much less likely to be a lethal mutation. As a result, there are 18 possible amino acid substitutions with leading to ALS-resistance in weed species and six leading to ACC-ase resistant grass weeds (TRANEL and WRIGHT, 2002; DELYE, 2005).

Management Factors

Management is the one aspect of the weed-herbicide-management triangle where the grower can influence herbicide resistance development. A grower that is able to implement integrated weed management tools such as crop rotation, cultivation, cover crops, and optimal agronomic management will put less selection pressure for the evolution of herbicide-resistant weeds than the grower who relies solely on herbicides for weed control or even the grower who uses some herbicide-resistance management strategies such as rotating herbicide mechanisms of action but does not use non-herbicide management tools. Cultural control measures relevant to arable and horticultural crops include crop rotation, mechanical weed control, competitive seeding rates, optimal row spacing, and preventing seed return to the weed seedbank.

If the herbicide is likely to be used several times per year (e.g. intensively managed crops), the risk is likely to be higher than where it is used once annually (as in successive cereal crops). The risk is likely to be lower where the herbicide is used less than once per year. If the herbicide is used alone, then the risk is likely to be higher than if it is used in mixture or sequence with other herbicides with activity on the same target weed, as long as the alternative herbicides have different mechanism of action (DIGGLE *et al.,* 2003). When the herbicide is the only one available for controlling a specific weed, the resistance risk is likely to be higher than if many alternative mechanism of action available.

If other weed management tools are not used, the risk of herbicide resistance increases. For example, incidences of herbicide resistance increase in minimum-tillage and no-till systems due to greater herbicide use and/or more rapid weed seedbank turnover than is found in conventional tillage (BECKIE, 2009). Weed resistance was greater in low soil disturbance no-till systems (BECKIE *et al.,* 2008).

Cropping system diversity is a cornerstone of weed resistance management. The risk of herbicide resistance is greatest in monoculture fields. In western Canada, the risk is lowest in cropping systems that include forage crops (tillage required to terminate the crop), fallow, or have three or more crop types (BECKIE, 2009). Diversity in weed management tactics is the single most important tactic for reducing and managing the evolution of herbicide-resistant weeds. Overreliance on a single herbicide or single group of herbicides without concurrent utilization of other weed management strategies has encouraged evolution of weed populations resistant to the heavily used herbicide(s); but it is not the only factor. Characteristics of the herbicide and herbicide class,

weed biology, and cultural practices such as crop rotation, tillage practices, and time of planting all play a role in determining the likelihood and frequency of herbicide resistance. Resistance to herbicides is a function of 1) the frequency of herbicide use; 2) how the herbicide has been used; 3) the strong selection pressure that is characteristic of the herbicide(s), and 4) the resistance mechanism in the weed - that is, whether changes to the target site occur easily without affecting plant function (e.g. ALS), or whether the target site is highly conserved so that occurrence of target-site resistance is difficult because of the negative effects on the plant (TRANEL and WRIGHT, 2002).

Resistance Risk Framework

Table 2 contains a resistance risk classification table that lists a ranked risk of herbicide resistance development for a given matrix of herbicide site of action, weed, and general weed management strategy. Weeds were ranked on a scale of one to three (three exhibiting the greatest and one the least risk) based on the prevalence of resistance development for the weed species and/or genus. Herbicides were ranked on a scale of one to three based on the prevalence of weeds to develop resistance to a particular target site. Finally, three general weed management systems were considered in regards to their influence of herbicide resistance development. The three weed management systems included in the matrix on a scale of 0.75 to one with one having the highest risk of herbicide resistance development. The weed management systems ran from reliance on a single herbicide mechanism of action for weed control to a herbicide based system relying on rotation of herbicide mechanisms of action. The lowest risk weed management system (0.25) is an integrated weed management system that uses herbicide site of action rotation, crop rotation, mechanical weed control, and cultural weed control tools.

In the resistance risk matrix, any single cell is a product of the individual weed by herbicide by weed management risks. A higher matrix product indicates a greater risk of herbicide resistance development. For example, the use of an ALS-inhibitor for *A. palmeri* control without herbicide rotation or any integrated weed management would result in a score of nine, the highest possible. In this situation, the risk of resistance development is extremely high and a grower or one advising a grower should advise an immediate change in the weed control strategy or regulatory officials approving the approval of a herbicide for such a situation should mandate at minimum proper notices of the risk of herbicide resistance development or not approve the use in some instances. It could be argued in these cases, the evolution of resistance is inevitable if the herbicide is applied. For those scenarios with a matrix product of three to four, resistance can possibly be delayed for a considerable time period if intensive resistance management strategies are employed (e.g. integrated weed management). On the other end of the scale, any matrix product less than three should not greatly concern the grower or other interested parties in resistance development.

Tab. *2* Herbicide resistance risk framework.

Tab. 2 *Risikorahmen der Herbizidresistenz.*

					Highest Risk (9) Higher Risk (6) Moderate Risk (3-4) Low Risk (0.125-2.25)	
High – ALS, ACC, PS-II-T	3	3	6	9	1 (High)	Single Herbicide MOA
		1.5	3.0	4.5	0.5 (Moderate – Low)	MOA Rotation only
		0.75	1.5	2.25	0.25 (Low)	Integrated Pest Mgmt
Moderate – EPSP, Auxin, DNA, PS-I, PDS	2	2.0	4	6	1 (High)	Single Herbicide MOA
		1.0	2	3.0	0.5 (Moderate – Low)	MOA Rotation only
		0.5	1	1.5	0.25 (Low)	Integrated Pest Mgmt
Low – VLCFA, GS	1	0.5	1	3.0	1 (High)	Single Herbicide MOA
		0.25	0.5	1.5	0.5 (Moderate – Low)	MOA Rotation only
		0.125	0.25	0.75	0.25 (Low)	Integrated Pest Mgmt
		1	2	3		
		ELEIN, *Senecio*, SOLXX, *Salsola, Poa*, DIGXX	*Avena*, ECHCG, XANST, SORHA, SETXX	AMAXX, AMBXX, LOLXX, KOCXX, CHEAL, *Conyza*		

In conclusion, the development of herbicide resistance is the product of weed, herbicide, and management factors present in a given field. If provided with predictive tools, growers will be better able to make decisions to delay the development of herbicide resistance. It is the duty of weed scientists to provide predictive tools that allow growers to know if they have certain weeds present, that they will see fairly rapid herbicide resistance development if they do not use an intensive regime of integrated weed management to reduce the need for herbicide applications.

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